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# Combined effects of phosphorus nutrition and elevated carbon dioxide concentration on chlorophyll fluorescence, photosynthesis, and nutrient efficiency of cotton

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## Abstract

To examine the combined effects of phosphorus (P) nutrition and $CO_2$ on photosynthesis, chlorophyll fluorescence (CF), and nutrient utilization and uptake, two controlled-environment experiments were conducted using 0.01, 0.05 and 0.20 mM external phosphate each at ambient and elevated $CO_2$ ($aCO_2$: 400 and $eCO_2$: 800 μmol $mol^{-1}$, respectively). The CF parameters were affected more by P nutrition than by $CO_2$ treatment. Photoinhibition of photosystem II (PSII) was due to increased minimal CF (Fo′) and decreased maximal CF (Fm′), and efficiency of energy harvesting (Fv′/Fm′). In addition, reduced electron transport rate (ETR), the quantum yield of PSII ($\Phi_{PSII}$) and $CO_2$ assimilation ($\Phi_{CO_2}$), and overall photochemical quenching in the P-deficient leaves led to reduction in the efficiency of energy transfer to the PSII reaction center. Stimulation in the $\Phi_{PSII}/\Phi_{CO_2}$ and photorespiration ($ETR/P_{net}$) was found under P deficiency, whereas the opposite was the case under $CO_2$ enrichment. On average, photosynthetic rate ($P_{net}$) and stomatal conductance declined by 50–53% at 0.05 mM P and by 70–72% at 0.01 mM P as compared to the 0.20 mM P treatment. However, P deficiency, especially at $eCO_2$, tended to increase the intrinsic water-use efficiency. In the P-deficient plants, the decline in the P and N utilization efficiency (up to 91%) of biomass production was mainly associated with greater reduction in the biomass relative to the tissue P concentration as the P supply was reduced. However, it was significantly stimulated by $eCO_2$ especially at higher P supply. The $CO_2 \times P$ interaction was observed for some parameters such as Fo′, Fm′, P utilization efficiencies of photosynthesis and biomass production that might be attributed to the irresponsiveness of these parameters to $eCO_2$ under low P treatment. Thus, P deficiency limited the beneficial effect of $eCO_2$. A close relationship between total biomass and photosynthesis with the P and N utilization or uptake efficiencies was found. The P utilization efficiency of $P_{net}$ appeared to be stable across a range of leaf P concentrations, whereas the N-utilization efficiency markedly increased with leaf P and differed between $CO_2$ levels. An apparent effect of both the treatments (P and $CO_2$) on N-uptake and utilization efficiency also indicated the alteration in N acquisition and assimilation in cotton plants.

**Key words:** *Gossypium hirsutum* / nitrogen / photochemical quenching / photosystems

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## 1 Introduction

Cotton (*Gossypium hirsutum* L.) is one of the most important fiber crops grown worldwide (*FAO*, 2007). Phosphorus deficiency inhibits cotton growth and development by decreasing leaf and stem expansion, photosynthesis, and total dry matter accumulation leading to lower crop yield (*Singh* et al., 2006). The ambient atmospheric $CO_2$ ($aCO_2$) of approximately 400 μmol $mol^{-1}$ is estimated to reach 730–1020 μmol $mol^{-1}$ by the end of 21st century (*IPCC*, 2007). In general, plants respond positively to an increase in $CO_2$ concentration, particularly $C_3$ crop species including cotton. While crop productivity may benefit from rising $CO_2$, the interactions with other abiotic factors including plant nutritional status may limit the beneficial effects of the elevated $CO_2$ ($eCO_2$) on crop growth and yield (*Campbell* and *Sage*, 2006; *Reddy* et al., 2004; *Reddy* et al., 1997; *Singh* et al., 2010).

Gas exchange and chlorophyll fluorescence are integral parts of photosynthetic processes in leaves. In the light reaction, the photosynthetic pigments absorb the light energy which is funneled through a functional array of Photosystem II (PSII) and Photosystem I (PSI) to generate chemical energy that will be used for $CO_2$ fixation in the dark reaction. The absorbed light energy that exceeds the photochemical processes of $CO_2$ fixation can be either dissipated as heat or re-emitted as chlorophyll fluorescence (*Maxwell* and *Johnson*, 2000). The maximum photosynthetic activity depends upon the capacity of electron transport (energy transfer) and the metabolically available phosphorus concentration in the chloroplast to maintain optimum photophosphorylation and biosynthesis of carbohydrates (*Conroy* et al., 1986). Increased utilization of the absorbed light energy in the dark reaction leads to a decrease in chlorophyll fluorescence yield. Detection of chlorophyll fluorescence from PSII provides information on the per-

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formance of the photosystem and may reflect overall photosynthetic activity (*Maxwell* and *Johnson*, 2000; *Roháček*, 2002). Environmental stresses such as nutrient deficiency decrease the performance of the photosystem mainly by damaging the PSII, making chlorophyll fluorescence a valuable tool to detect the influence of stress factors on plant photosynthesis (*Bown* et al., 2009; *Maxwell* and *Johnson*, 2000; *Shao* et al., 2013; *Singh* et al., 2013a). The series of events that take place during the induction of leaf chlorophyll fluorescence have been detailed previously by *Maxwell* and *Johnson* (2000), *Genty* et al. (1989) and *Roháček* (2002).

The rate of photosynthesis in many $C_3$ plants is limited by the current atmospheric $CO_2$ concentration. Moreover, the growth stimulation by elevated $CO_2$ is expected to increase the overall plant demand for mineral nutrients such as nitrogen (N) and phosphorus (P). Elevated $CO_2$ tends to increase the total nutrient accumulation in plants but decreases their tissue concentration leading to alterations in nutrient uptake and use efficiency (*Lenka* and *Lal*, 2012; *Rogers* et al., 1993; *Singh* et al., 2013b). The critical leaf tissue concentration (defined as concentration to achieve 90% of maximum productivity) of N often decreases under $eCO_2$. However, this is not always true for P (*Conroy*, 1992; *Conroy* et al., 1990; *Rogers* et al., 1993). Compared to N nutrition, the interactions between P and $CO_2$ have not been explored much in agronomic crops, but limited studies indicated that higher foliar concentrations of P may be needed for maximum growth in many agronomic crops and trees (*Conroy* et al., 1990; *Rogers* et al., 1993).

Mineral nutrition should take into account both nutrient availability and tissue concentration. Large uncertainty exists about the changes in the mineral availability and the response of P concentration under $eCO_2$ conditions (*Sinclair*, 1992). Nutrient acquisition and assimilation by plants are strongly influenced by $eCO_2$. Elevated $CO_2$-mediated decreases in tissue nutrient concentrations have commonly been observed in many crops grown under similar nutrient supply at $aCO_2$ (*Conroy*, 1992; *Cure* et al., 1988; *Singh* et al., 2013b). However, the decreases in tissue nutrient concentration under $eCO_2$ were not associated with a limitation of growth or photosynthetic processes but increased the nutrient utilization efficiency especially for N (*Barrett* and *Gifford*, 1995; *Prior* et al., 1998; *Singh* et al., 2013a). The beneficial effect of $eCO_2$ on plant growth has been shown to decline under N-limited conditions. Such studies with P are limited in row crops such as cotton. Therefore, it is important to understand the interactive effect of P and $CO_2$ on crop growth and nutrient uptake and utilization (*Sinclair*, 1992).

The tissue nutrient concentration represents plant nutrient status and is used in the estimation of utilization efficiency in biomass production (*Israel* and *Rufty*, 1988; *Siddiqi* and *Glass*, 1981). Plants may be more efficient in nutrient acquisition and utilization under $eCO_2$ due to increased growth and decreased tissue concentration (*Pérez-López* et al., 2014; *Prior* et al., 1998; *Rogers* et al., 1993). However, under P-limited supply this response of $eCO_2$ may be limited. Previous studies also suggested an increased P and N utilization efficiency of plants when grown under nutrient deficiency (*Cure* et al., 1988; *Prior* et al., 1998). Therefore, the $eCO_2$ may alter the nutrient dynamics inside the plants, which might be escalated under P deficiency. Therefore, the objectives of this study were to determine the changes in chlorophyll fluorescence, gas exchange, and P and N utilization and uptake efficiencies of cotton grown with different levels of P supply under ambient and elevated $CO_2$ concentration. We hypothesize that chlorophyll fluorescence reflects changes in the photosynthetic efficiency by a decrease due to P deficiency or increase due to $eCO_2$. Additionally, the P and N utilization efficiencies will be favored by both P deficiency and $eCO_2$ in cotton.

## 2 Material and methods

Two experiments (Experiment 1 and Experiment 2) were conducted using six controlled environmental growth chambers (EGC Corp., Chagrin Falls, OH, USA) at USDA-ARS facility in Beltsville, MD, USA. In Experiment 1, cotton (*Gossypium hirsutum* L. cv. deltapine 555) seeds were planted in twelve pots (16 L) filled with fine sand and vermiculate (3 : 1 volume ratio) in six growth chambers. Two levels (400 and 800 μmol $mol^{-1}$) of $CO_2$ treatments were initiated each in three chambers from the planting and plants were watered with full-strength Hoagland's nutrient solution (*Hewitt*, 1952) from emergence to 34 d after planting (DAP). Thereafter, three P treatments were initiated by irrigating with modified Hoagland's nutrient solution (0.01, 0.05, and 0.20 mM $KH_2PO_4$) at each of the two $CO_2$ levels. Plants were irrigated using automated drip irrigation until the pots started to flush from the bottom three or five times a day.

In Experiment 2, the same cultivar was first planted outdoor in pots (7.6 L) containing rooting medium similar to Experiment 1. Plants were irrigated with the same nutrient solution as in the previous experiment from emergence to 34 DAP. Thereafter, plants were transferred and randomly assigned to the six growth chambers (20 pots each), and the treatments were initiated as in Experiment 1.

In both experiments, one plant per pot was maintained. A 30/22°C day/night (14 h day and 10 h night) air temperature was maintained during experiments. The photosynthetically active radiation (PAR) of 800 μmol $m^{-2}$ $s^{-1}$ was maintained at plant canopy using a mixture of metal halide and high-pressure sodium lamps during the day. The desired $CO_2$ concentrations inside the growth chambers were maintained by injecting either $CO_2$ or $CO_2$-free air. The $CO_2$-free air ($CO_2$ < 1 μmol $mol^{-1}$) was obtained using a Parker Balston FT-IR Purge Gas Generator Model # 75–62 (Parker Hannifin Corporation, Haverhill, MA, USA). The relative humidity varied between 50 and 70% during the experiment among the chambers. The main differences between the above two experiments were initiation of $CO_2$ treatments, number and size of the pots, and time and frequency of the measurements. Other details of plant growth conditions are given in *Singh* et al. (2013b).

### 2.1 Plant biomass

Plants were harvested at 84 and 112 DAP (six pots each) in Experiment 1, and 67, 81, and 91 DAP (six, six and eight pots, respectively) in Experiment 2. Roots were washed in clean water. Plants were separated into roots, stems and

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leaves. The dry weights of plant materials were determined after drying in an oven at 70°C for 10 d until constant weight was obtained.

### 2.2 Gas exchange and chlorophyll fluorescence measurements

In both experiments, periodically from 57 to 112 DAP, the gas exchange and chlorophyll fluorescence (CF) parameters were measured simultaneously on the upper most fully expanded leaves between 9:00 and 13:00 h from three to five individual plants per treatment using a LI–6400 (LI–6400 portable photosynthesis system, LI-COR Inc., Lincoln, NE) with an integrated fluorescence chamber head (LI-COR 6400–40 Leaf Chamber Fluorometer; Li-Cor Inc.). The leaf temperature was set to the day-time air temperature (28°C) and $CO_2$ was controlled by the $CO_2$ injection system to match the $CO_2$ treatments. The PAR, provided by a 6400-02 LED light source, was set to 1500 μmol $m^{-2}$ $s^{-1}$. Relative humidity inside the cuvette varied between 45 and 60%. To measure fluorescence, the built-in leaf chamber fluorometer was used which uses two red LEDs (center wavelength about 630 nm) and a detector (for radiation at 715 nm in the PSII fluorescence band). A flash light (> 8000 μmol $m^{-2}$ $s^{-1}$), achieved by using 27 red LEDs, was used to measure the maximal fluorescence (Fm′). Rapid dark adaptation to measure minimal fluorescence (Fo′) was achieved by turning off the actinic light while using the far-red LED (center wavelength at 740 nm). The far red radiation drives photosystem-I (PSI) momentarily to help drain electrons from PSII. The response of CF parameters to photosynthetically active radiation (PAR) and internal $CO_2$ concentration ($C_i$) was also measured between 69 and 75 d after planting in Experiment 2 using the above-mentioned Li-COR photosynthesis system according to *Singh* et al. (2013a). The rate of net photosynthesis ($P_{net}$), stomatal conductance ($g_s$), intrinsic water-use efficiency (WUE, $P_{net}/g_s$), quantum efficiency by oxidized (open) PSII reaction center in light (Fv′/Fm′), actual flux of photons driving photosystem II (PSII), *i.e.*, electron transport rate (ETR), photochemical yield of PSII electron transport rate ($\Phi_{PSII}$), and quantum yield of $CO_2$ fixation ($\Phi_{CO_2}$) were automatically computed from the instrument software (details are available in LI–6400 Instruction Manual, version 5, Li-Cor Inc., Lincoln, Nebraska, USA).

### 2.3 Measurement of SPAD chlorophyll meter reading (SCMR) and tissue phosphorus and nitrogen concentration

In both experiments, the SCMR (an indicator of chlorophyll concentration), tissue P and N concentrations were determined from the same leaves that were used for gas-exchange measurements. The SCMR was taken using a SPAD 502 Plus Chlorophyll Meter (Spectrum Technologies, Inc, Aurora, IL, USA). The tissue P and N concentrations were also measured in plant organs (leaves, stems and roots) of each harvest. The dry plant materials were ground using a Wiley Mill (Wiley® Mill, Thomas Scientific, NJ, USA) to pass through 1 mm screen. The tissue P concentration was quantified in the Soil Testing and Plant Analysis Laboratory, Extension Service, Mississippi State University, MS, USA. The N concentration was determined by combustion using a CHN–2000 (Carbon Hydrogen Nitrogen–2000: LECO Corporation, St. Joseph, MI, USA). The weighted whole-plant P and N concentrations were estimated as the sum of the products of dry mass of plant organs and their nutrient concentration divided by total biomass.

### 2.4 Nutrient utilization and uptake efficiency

The P and N utilization efficiencies for biomass production ($PUE_{Bio}$ and $NUE_{Bio}$) were estimated by dividing total biomass by the whole-plant P or N concentration. The P and N utilization efficiencies for $P_{net}$ ($PUE_{Pnet}$ and $NUEP_{net}$) were calculated by dividing $P_{net}$ by P or N concentration of the same leaf. The total P or N absorbed (mg $plant^{-1}$) per unit of root biomass was used as an indicator of P or N uptake efficiency (PUpE or NUpE).

### 2.5 Data analysis

Statistical analyses were performed using SAS procedures (SAS Enterprise Guide, 4.2, SAS Institute Inc., NC, USA). To test the effect of treatments and their interaction, PROC MIXED with Kenward–Rogers (kr) adjustment of degrees of freedom was used for analysis of variance (ANOVA). The measurements from the individual plants were treated as a replication for each chamber. Treatments (P and $CO_2$) and their interaction were considered as the fixed and chamber as the random effect. The normality assumptions were assessed using Sharpio–Wilks statistics and log transformation was used if necessary. Any heterogeneity in the data was corrected using the GROUP option in the REPEATED statement of the ProcMixed procedure. The treatment comparisons were conducted by least square means (LSMEANS) procedure (at $P$ = 5%) with the letter grouping obtained using pdmix800 macro (*Saxton*, 1998). ProcMixed procedure of SAS was also used for regression analysis and to test for the common slopes ($P$ = 5%) between $CO_2$ levels. In both experiments, ANOVA was used separately for each harvest. Since there were no significant differences for leaf level measurements between the two experiments, the data measured on uppermost fully expanded leaves were combined.

## 3 Results

### 3.1 Chlorophyll fluorescence and photosynthetic processes

Regardless of $CO_2$ levels, P deficiency reduced Fm′ (25–36%), Fv′/Fm′ (41–51%), qP (16–22%), ETR and $\Phi_{PSII}$ (49–61%), and $\Phi_{CO_2}$ (54–80%), whereas it increased Fo′ (1–16%), $\Phi_{PSII}/\Phi_{CO_2}$ (13–38%), and $ETR/P_{net}$ (21–84%; Table 1). Elevated $CO_2$ did not show a significant effect on Fo′ and Fm′. However, it reduced other CF parameters except the $\Phi_{CO_2}$ when averaged across P treatments. The $eCO_2$-mediated decrease in Fv′/Fm′, ETR, and $\Phi_{PSII}$ was highest at the lowest P treatment, whereas the decrease in $\Phi_{PSII}/\Phi_{CO_2}$ and $ETR/P_{net}$ was greatest in the 0.05 mM P treatment. Regardless of the treatments, the qP, Fo′, Fm′, Fv′/Fm′, and $\Phi_{PSII}$ decreased with increase in PAR, whereas ETR in-

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**Table 1**: Effect of $CO_2$ levels (μmol mol$^{-1}$) and phosphate (P; mM) supply on minimal (Fo′) and maximal (Fm′) fluorescence of light-adapted leaves, the efficiency of energy harvesting by oxidized (open) PSII reaction centers in light (Fv′/Fm′), photochemical quenching (qP), electron transport rate (ETR; μmol electron m$^{-2}$ s$^{-1}$), fraction of absorbed photons that are used for dark reaction of a light-adapted leaf [$\Phi_{PSII}$, also called as effective quantum yield; μmol electron (μmol photon)$^{-1}$], apparent quantum yield of $CO_2$ assimilation [$\Phi_{CO_2}$; μmol $CO_2$ (μmol photon)$^{-1}$], $\Phi_{PSII}/\Phi_{CO_2}$ ratio, ETR/net photosynthetic rate (ETR/$P_{net}$; μmol electron μmol$^{-1}$ $CO_2$), SPAD chlorophyll meter reading (SCMR), and intrinsic water use efficiency (WUE; μmol $CO_2$ mol$^{-1}$ $H_2O$) of uppermost fully expanded leaves of cotton. The *P* values of the analysis of variance (ANOVA) between P and $CO_2$ are given. The data are means of 13 d measurements between 57 and 112 d after planting across two experiments. Within column, means followed by same letters are not significantly different at *P* = 5%.

| $CO_2$ | P | Fo′ | Fm′ | Fv′/Fm′ | qP | ETR | $\Phi_{PSII}$ | $\Phi_{CO_2}$ | $\Phi_{PSII}/\Phi_{CO_2}$ | ETR/$P_{net}$ | SCMR | WUE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 400 | 0.20 | 475.3$^{d}$ | 1082.0$^{a}$ | 0.56 | 0.674 | 246.3 | 0.376 | 0.0209$^{b}$ | 18.36$^{b}$ | 9.77$^{b}$ | 53.56 | 75.63$^{d}$ |
| | 0.05 | 519.2$^{bc}$ | 776.4$^{b}$ | 0.33 | 0.566 | 122.2 | 0.186 | 0.0085$^{d}$ | 22.18$^{a}$ | 12.64$^{a}$ | 41.11 | 82.13$^{c}$ |
| | 0.01 | 553.4$^{a}$ | 788.2$^{b}$ | 0.29 | 0.529 | 103.2 | 0.157 | 0.0075$^{d}$ | 21.63$^{a}$ | 12.39$^{a}$ | 37.64 | 66.93$^{d}$ |
| 800 | 0.20 | 509.9$^{c}$ | 1095.4$^{a}$ | 0.53 | 0.628 | 218.3 | 0.332 | 0.0237$^{a}$ | 14.21$^{c}$ | 7.36$^{c}$ | 47.31 | 122.77$^{b}$ |
| | 0.05 | 538.4$^{ab}$ | 822.2$^{b}$ | 0.34 | 0.507 | 112.5 | 0.172 | 0.0109$^{c}$ | 16.03$^{c}$ | 8.91$^{bc}$ | 36.92 | 137.70$^{b}$ |
| | 0.01 | 514.7$^{c}$ | 695.7$^{c}$ | 0.26 | 0.489 | 82.5 | 0.127 | 0.0069$^{d}$ | 19.63$^{b}$ | 13.55$^{a}$ | 35.81 | 164.32$^{a}$ |
| *ANOVA* | $CO_2$ | 0.4445 | 0.4701 | 0.0412 | < 0.0001 | < 0.0001 | < 0.0001 | 0.0015 | < 0.0001 | 0.0026 | < 0.0001 | < 0.0001 |
| | P | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | 0.0464 |
| | $CO_2 \times$ P | < 0.0001 | 0.0009 | 0.0934 | 0.6799 | 0.2484 | 0.2189 | 0.0061 | 0.0111 | 0.0011 | 0.1174 | 0.0011 |

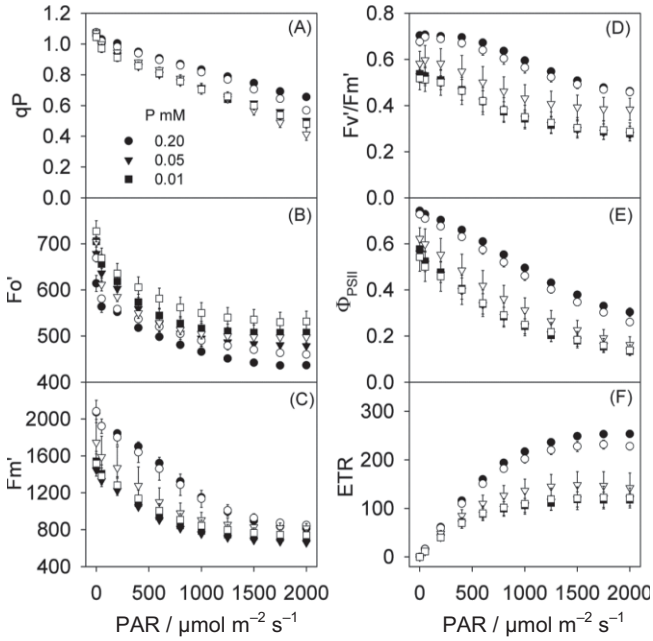


**Figure 1:** Response of photochemical quenching (qP), minimal (Fo′) and maximal (Fm′) fluorescence of light-adapted leaves, the efficiency of energy harvesting by oxidized (open) PSII reaction centers in light (Fv′/Fm′), the fraction of absorbed photons that are used for photochemistry for a light-adapted leaf [$\Phi_{PSII}$, also called as effective quantum yield; μmol electron (μmol photon)$^{-1}$], and electron transport rate (ETR, μmol electron m$^{-2}$ s$^{-1}$) to photosynthetically active radiation (PAR; μmol m$^{-2}$ s$^{-1}$) measured between 69 and 75 d after planting in uppermost fully expanded leaves of cotton grown at either ambient (filled symbols: 400 μmol mol$^{-1}$) or elevated (unfilled symbols: 800 μmol mol$^{-1}$) $CO_2$ under different phosphate (P) treatments. Error bars represent the standard errors of three to four replicates.

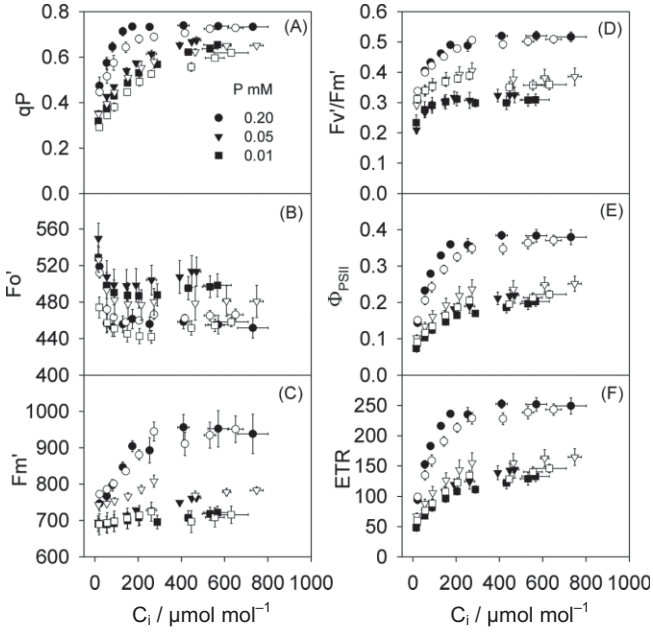


**Figure 2:** Response of photochemical quenching (qP), minimal (Fo′) and maximal (Fm′) fluorescence of light-adapted leaves, the efficiency of energy harvesting by oxidized (open) PSII reaction centers in light (Fv′/Fm′), the fraction of absorbed photon that are used for photochemistry of a light-adapted leaf [$\Phi_{PSII}$, also called as effective quantum yield; μmol electron (μmol photon)$^{-1}$], and electron transport rate (ETR; μmol electron m$^{-2}$ s$^{-1}$) to internal $CO_2$ concentration ($C_i$; μmol mol$^{-1}$) measured between 69 and 75 d after planting in uppermost fully expanded leaves of cotton grown at either ambient (filled symbols: 400 μmol mol$^{-1}$) or elevated (unfilled symbols: 800 μmol mol$^{-1}$) $CO_2$ under different phosphate (P) treatments. Error bars represent the standard errors of three to four replicates.

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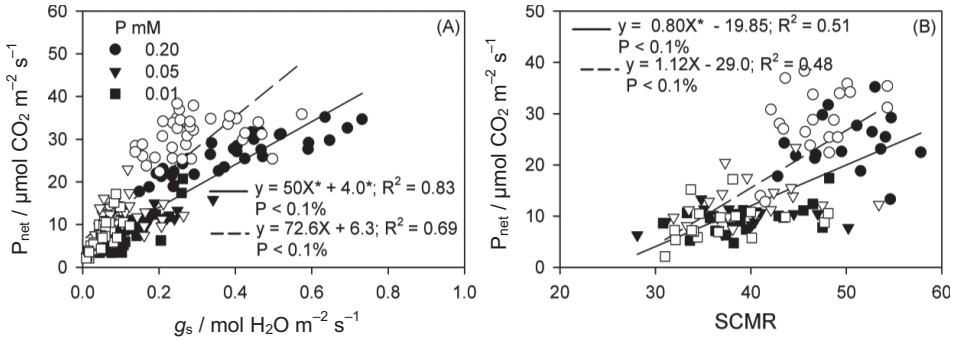


**Figure 3:** Relationship of net photosynthetic rate ($P_{net}$) with stomatal conductance ($g_s$) and SPAD chlorophyll meter reading (SCMR) for upper most fully expanded cotton leaves grown at either ambient (filled symbols and solid lines: 400 μmol mol$^{-1}$) or elevated (unfilled symbols and dashed lines: 800 μmol mol$^{-1}$) $CO_2$ under different phosphate (P) treatments. Data are from individual plants measured between 57 and 112 d after planting across two experiments. The coefficients of the linear regression analysis are also given. The asterisk (*) indicates significant ($P < 5\%$) difference for the given coefficient between $CO_2$ levels.

creased with PAR (Fig. 1). These CF parameters, except Fo′, increased with $C_i$ and the 0.20 mM treated plants showed the highest values across $CO_2$ treatments (Fig. 2). The CF parameters tended to be lower under $eCO_2$ *versus* $aCO_2$ especially at the 0.20 mM P in response to both PAR and $C_i$.

The phosphate deficiency exhibited a similar decline in $P_{net}$ and $g_s$ across $CO_2$ treatments (Fig. 3A). Averaged across all measurements, $P_{net}$ and $g_s$ declined by 50–53% at 0.05 mM P and by 70–72% at 0.01 mM P as compared to the 0.20 mM P treatment. The slope of the linear regression of $P_{net}$ with $g_s$ and SCMR differed between $CO_2$ levels (Fig. 3). The $eCO_2$ increased $P_{net}$ by 13.33% and decreased $g_s$ by 33% when averaged across P treatments. The SCMR declined significantly by 22–30% in P-deficient leaves across $CO_2$ levels (Table 1). SCMR was also lower (4.8–11.65%) at $eCO_2$ *versus* $aCO_2$. The WUE exhibited significant $CO_2 \times P$ interaction. The WUE tended to be higher under low P and at the $eCO_2$ except at the lowest P treatment (Table 1).

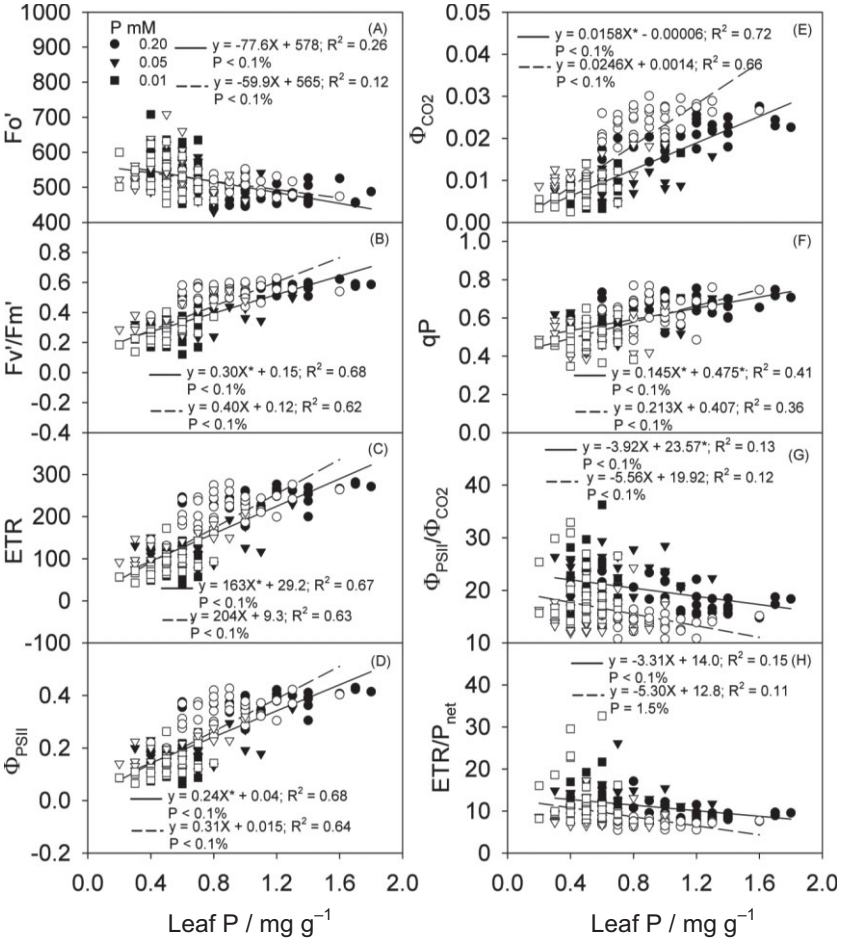


**Figure 4:** Relationship of leaf tissue phosphorus concentration (P) with minimal fluorescence (Fo′), the efficiency of energy harvesting by oxidized (open) PSII reaction centers in light (Fv′/Fm′), electron transport rate (ETR; μmol electron m$^{-2}$ s$^{-1}$), the fraction of absorbed photon that are used for photochemistry for a light adapted leaf [$\Phi_{PSII}$, μmol electron (μmol photon)$^{-1}$], the apparent quantum yield of $CO_2$ assimilation [$\Phi_{CO_2}$, μmol $CO_2$ (μmol photon)$^{-1}$], photochemical quenching (qP), $\Phi_{PSII}/\Phi_{CO_2}$ ratio, and the ratio of ETR/net photosynthetic rate (ETR/$P_{net}$; μmol electron μmol$^{-1}$ $CO_2$) for upper most fully expanded cotton leaves grown at either ambient (filled symbols and solid lines: 400 μmol mol$^{-1}$) or elevated (unfilled symbols and dashed lines: 800 μmol mol$^{-1}$) $CO_2$ under different phosphate (P) treatments. Data are from individual plants measured between 57 and 112 d after planting across two experiments. The coefficients of the linear regression analysis are also given. The asterisk (*) indicates significant ($P < 5\%$) difference for the given coefficient between $CO_2$ levels.

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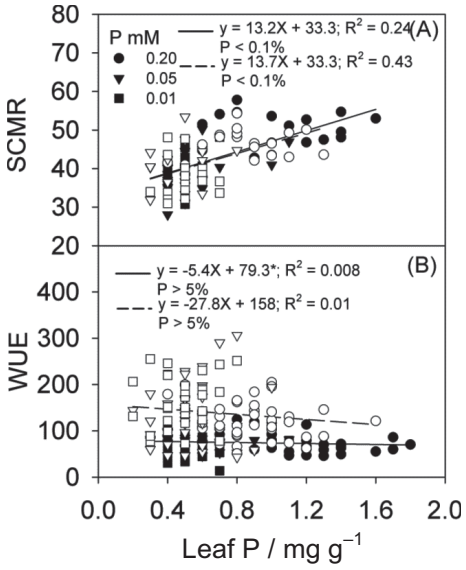


**Figure 5:** Relationship of leaf tissue phosphorus concentration (P) with SPAD chlorophyll meter reading (SCMR) and intrinsic water-use efficiency (WUE; μmol $CO_2$ $mol^{-1}$ $H_2O$) for upper most fully expanded cotton leaves grown at either ambient (filled symbols and solid lines: 400 μmol $mol^{-1}$) or elevated (unfilled symbols and dashed lines: 800 μmol $mol^{-1}$) $CO_2$ under different phosphate (P) treatments. Data are from individual plants measured between 57 and 112 d after planting across two experiments. The coefficients of the linear regression analysis are also given. The asterisk (*) indicates significant ($P < 5\%$) difference for the given coefficient between $CO_2$ levels.

### 3.2 Relationship between tissue P concentration and photosynthetic parameters

The tissue P concentration exhibited a significant linear relationship with many of the studied parameters excluding one with WUE (Fig. 4 and 5). The leaf P concentration also showed a significant linear increasing effect on Fo′, Fv′/Fm′, ETR, $\Phi_{PSII}$, qP, and $\Phi_{CO_2}$ (Fig. 4A–F). In contrast, the ratio of $\Phi_{PSII}/\Phi_{CO_2}$ and $ETR/P_{net}$ significantly increased as leaf P concentration decreased, and the values tended to be higher at $aCO_2$ *versus* $eCO_2$ (Fig. 4G–H). The SCMR increased with leaf P concentration and the slope between $CO_2$ levels did not differ for SCMR *versus* leaf P (Fig. 5A). The relationship between WUE and the leaf tissue P concentration was not significant. However, WUE tended to be higher at $eCO_2$ *versus* $aCO_2$ (Fig. 5B).

### 3.3 Nutrient utilization and uptake efficiency

The $PUE_{Pnet}$ and $NUE_{Pnet}$ showed a significant $CO_2 \times P$ interaction and declined on averaged 33.40% in P-deficient *versus* 0.20 mM P-treated plants across $CO_2$ levels with greater decline at the lowest P (Table 2). Elevated $CO_2$ significantly increased $PUE_{Pnet}$ and $NUE_{Pnet}$ only at the two highest P levels. The P and N utilization efficiencies of biomass production exhibited significant treatment effects at all harvests in both the experiments (Table 2). In general, P deficiency reduced whereas $eCO_2$ increased $PUE_{Bio}$ and $NUE_{Bio}$ across experiments. Averaged across $CO_2$ treatments, the $PUE_{Bio}$ combined for the two low P treatments decreased by 87.14 and 65.0% in Experiment 1 and Experiment 2, respectively, as compared to 0.20 mM P at the final harvests. However, this decrease in $NUE_{Bio}$ was greater, *e.g.*, 91.34 and 79.29% in Experiment 1 and Experiment 2, respectively. Elevated $CO_2$ significantly stimulated both $PUE_{Bio}$ and $NUE_{Bio}$ especially at 0.20 mM P, and this stimulation was either small or none at the lowest P treatment (Table 2). Irrespective of the harvest dates in both experiments, the two low P treatments significantly decreased the averaged PUpE and NUpE of plants by 55–78% and 30–55%, respectively, across $CO_2$ levels (Table 3). There were strong linear correlations of $P_{net}$ with $PUE_{Pnet}$ and $NUE_{Pnet}$, and total biomass with $PUE_{Bio}$,

**Table 2**: Effect of $CO_2$ levels (μmol $mol^{-1}$) and phosphate (P; mM) supply on P and N utilization efficiency of biomass production ($PUE_{Bio}$ and $NUE_{Bio}$ respectively; $g^2$ dry weight $mg^{-1}$ P), and photosynthesis [$PUE_{Pnet}$ and $NUE_{Pnet}$, respectively; g (μmol $CO_2$ $m^{-2}$ $s^{-1}$) $mg^{-1}$ P]. The $PUE_{Pnet}$ and $NUE_{Pnet}$ data are combined across both experiments. The $PUE_{Bio}$ and $NUE_{Bio}$ data are the means of 6–8 plants for Experiment 1 (84 and 112 days after planting, DAP) and Experiment 2 (67 and 91 DAP). The *P* values of the analysis of variance (ANOVA) between P and $CO_2$ are given. Within columns, means followed by same letters are not significantly different at $P = 5\%$.

| | | | | Experiment 1 | | | | Experiment 2 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 84 DAP | | 112 DAP | | 67 DAP | | 91 DAP | |
| $CO_2$ | P | $PUE_{Pnet}$ | $NUE_{Pnet}$ | $PUE_{Bio}$ | $NUE_{Bio}$ | $PUE_{Bio}$ | $NUE_{Bio}$ | $PUE_{Bio}$ | $NUE_{Bio}$ | $PUE_{Bio}$ | $NUE_{Bio}$ |
| 400 | 0.20 | $23.25^{c}$ | $8.57^{b}$ | $41.61^{b}$ | $2.55^{b}$ | $110.77^{b}$ | $4.24^{b}$ | 15.77 | 0.57 | 74.79 | 2.43 |
| | 0.05 | $17.27^{d}$ | $3.33^{d}$ | $10.93^{c}$ | $0.36^{d}$ | $34.00^{c}$ | $0.86^{c}$ | 18.04 | 0.37 | 25.90 | 0.51 |
| | 0.01 | $16.01^{d}$ | $2.76^{d}$ | $9.69^{c}$ | $0.31^{d}$ | $10.06^{d}$ | $0.20^{e}$ | 14.36 | 0.26 | 24.96 | 0.54 |
| 800 | 0.20 | $33.95^{a}$ | $13.13^{a}$ | $73.87^{a}$ | $3.92^{a}$ | $222.16^{a}$ | $7.71^{a}$ | 25.77 | 0.92 | 90.48 | 4.09 |
| | 0.05 | $27.00^{b}$ | $5.45^{c}$ | $32.60^{b}$ | $1.12^{c}$ | $35.64^{c}$ | $0.83^{cd}$ | 18.30 | 0.40 | 31.85 | 0.98 |
| | 0.01 | $15.91^{d}$ | $3.20^{d}$ | $17.67^{c}$ | $0.71^{cd}$ | $5.92^{d}$ | $0.18^{de}$ | 12.34 | 0.28 | 32.76 | 0.67 |
| *ANOVA* | $CO_2$ | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | 0.2137 | 0.0306 | 0.0425 | 0.0263 |
| | P | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | 0.0351 | < 0.0001 | < 0.0001 | < 0.0001 |
| | $CO_2 \times P$ | < 0.0001 | < 0.0001 | 0.0065 | 0.0242 | < 0.0001 | < 0.0001 | 0.0740 | 0.0505 | 0.9494 | 0.1608 |

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**Table 3**: Effect of $CO_2$ levels (μmol mol$^{-1}$) and phosphate (P; mM) supply on P and N uptake efficiency ($PUpE_{Bio}$ and $NUpE_{Bio}$ respectively; mg P g$^{-1}$ root dry weight). The data are the means of 6–8 plants for Experiment 1 (84 and 112 d after planting, DAP) and Experiment 2 (67 and 91 DAP). The *P* values of the analysis of variance (ANOVA) between P and $CO_2$ are given. Within columns, means followed by same letters are not significantly different at *P* = 5%.

| | | Experiment 1 | | | | Experiment 2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 84 DAP | | 112 DAP | | 67 DAP | | 91 DAP | |
| $CO_2$ | P | PUpE | NUpE | PUpE | NUpE | PUpE | NUpE | PUpE | NUpE |
| 400 | 0.20 | 5.99 | 97.49$^{b}$ | 5.35 | 156.2 | 3.95 | 108.6 | 5.41$^{b}$ | 163.2 |
| | 0.05 | 2.62 | 78.71$^{bc}$ | 2.93 | 113.2 | 1.60 | 79.0 | 2.00$^{cd}$ | 99.9 |
| | 0.01 | 1.99 | 64.58$^{cd}$ | 1.06 | 43.6 | 1.47 | 73.8 | 1.71$^{cd}$ | 81.2 |
| 800 | 0.20 | 6.69 | 125.20$^{a}$ | 5.00 | 145.3 | 4.23 | 115.1 | 6.68$^{a}$ | 156.7 |
| | 0.05 | 2.61 | 80.30$^{bc}$ | 2.90 | 125.0 | 2.05 | 89.4 | 2.36$^{c}$ | 91.2 |
| | 0.01 | 1.85 | 44.80$^{d}$ | 1.24 | 48.4 | 1.65 | 70.8 | 1.37$^{d}$ | 47.5 |
| *ANOVA* | $CO_2$ | 0.4601 | 0.582 | 0.7219 | 0.7698 | 0.083 | 0.328 | 0.0564 | 0.0387 |
| | P | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| | $CO_2 \times$ P | 0.3361 | 0.0078 | 0.5184 | 0.3393 | 0.8014 | 0.5308 | 0.0255 | 0.2444 |

$NUE_{Bio}$, PUpE, and NUpE (*P* < 0.1%; Fig. 6). The regression slope across two levels of $CO_2$ did not differ for $P_{net}$ *versus* $PUE_{Pnet}$ and total biomass *versus* $PUE_{Bio}$ and PUpE (Fig. 6A, C, E). However, it differed significantly for and $P_{net}$ *versus* $NUE_{Pnet}$ and total biomass *versus* $NUE_{Bio}$ and NUpE, (Fig. 6 B, D).

### 3.4 Relationship between tissue-P concentration and nutrient-efficiency parameters

A significant relationship between leaf tissue P concentration and $PUE_{Pnet}$ was not observed. However, $PUE_{Pnet}$ appeared to be greater at $eCO_2$ *versus* $aCO_2$ (Fig. 7A). The $NUE_{Pnet}$ significantly increased with leaf P concentration and the response was also significantly higher at $eCO_2$ *versus* $aCO_2$ (Fig. 7B). The $PUE_{Bio}$ and $NUE_{Bio}$ exhibited a linear decline with tissue P concentrations and differed between $CO_2$ levels (Fig. 7 C, D). Elevated $CO_2$ exhibited a greater increase in $PUE_{Bio}$ and $NUE_{Bio}$ per unit increase of the tissue P concentration (Fig. 7 C, D). The PUpE and NUpE also decreased with tissue P concentration across both $CO_2$ levels (Fig. 7E, F).

## 4 Discussion

### 4.1 Chlorophyll fluorescence and photosynthetic parameters

In the P-deficient leaves, an increased Fo′ clearly indicated reduced efficiency of energy trapping at the PSII and the consequent inhibition of electron transfer as deduced from low Fv′/Fm′ and ETR. The increased Fo′ is related to the loss of chlorophyll (*i.e.*, SCMR) and disconnection of the light harvesting antennae from the PSII reaction center (*Conroy* et al., 1990; *Moseley* et al., 2002). The change in Fv′/Fm′ was associated with both an increased Fo′ and decreased Fm′, at which a low Fm′ suggested increased non-photochemical quenching due to enhanced mechanism of energy dissipation. This was supported by a concomitant decrease in qP leading to reduced $\Phi_{PSII}$ and $\Phi_{CO_2}$ (*Baker* and *Rosenqvist*, 2004). In fact, ETR and $\Phi_{PSII}$ showed a parallel decrease in response to low P supply across $CO_2$ levels as also deduced from the range of PAR and $C_i$ values (Fig. 1 and 2) indicating photoinhibition due to damage to the PSII reaction center. These CF parameters often responded to the increase in $C_i$ roughly up to 250 μmol mol$^{-1}$, thereafter saturation occurred.

The reduction in $g_s$ under $eCO_2$ has commonly been observed in many crops, which often results in reduced transpiration and increased WUE (*Reddy* et al., 1995; *Singh* et al., 2013a). This is in accordance with an observed increase in WUE under $eCO_2$. Phosphorus deficiency also increased the WUE except at the lowest P treatment under $aCO_2$ exhibiting a $CO_2 \times$ P interaction. The lower WUE under severe P stress at $aCO_2$ condition might be attributed to a greater reduction in $P_{net}$ than $g_s$ leading to lower carbon gain per unit loss of water during transpiration. The observed non-significant negative slope of WUE *versus* leaf P regression across $CO_2$ levels also showed that WUE tended to be higher at $eCO_2$, indicating cotton plants may be more efficient in water use under P deficiency when grown in $CO_2$ enriched environments (Fig. 5B). Decreases in $P_{net}$, $g_s$, and SCMR under P deficiency have been reported previously and attributed to the reduction in leaf chlorophyll and tissue P concentrations, and limitations caused in the $CO_2$ diffusion and photo-biochemical processes (*Singh* et al., 2013a). The functional relationship between $P_{net}$ and $g_s$ clearly indicated higher $P_{net}$ at a given $g_s$ under $eCO_2$.

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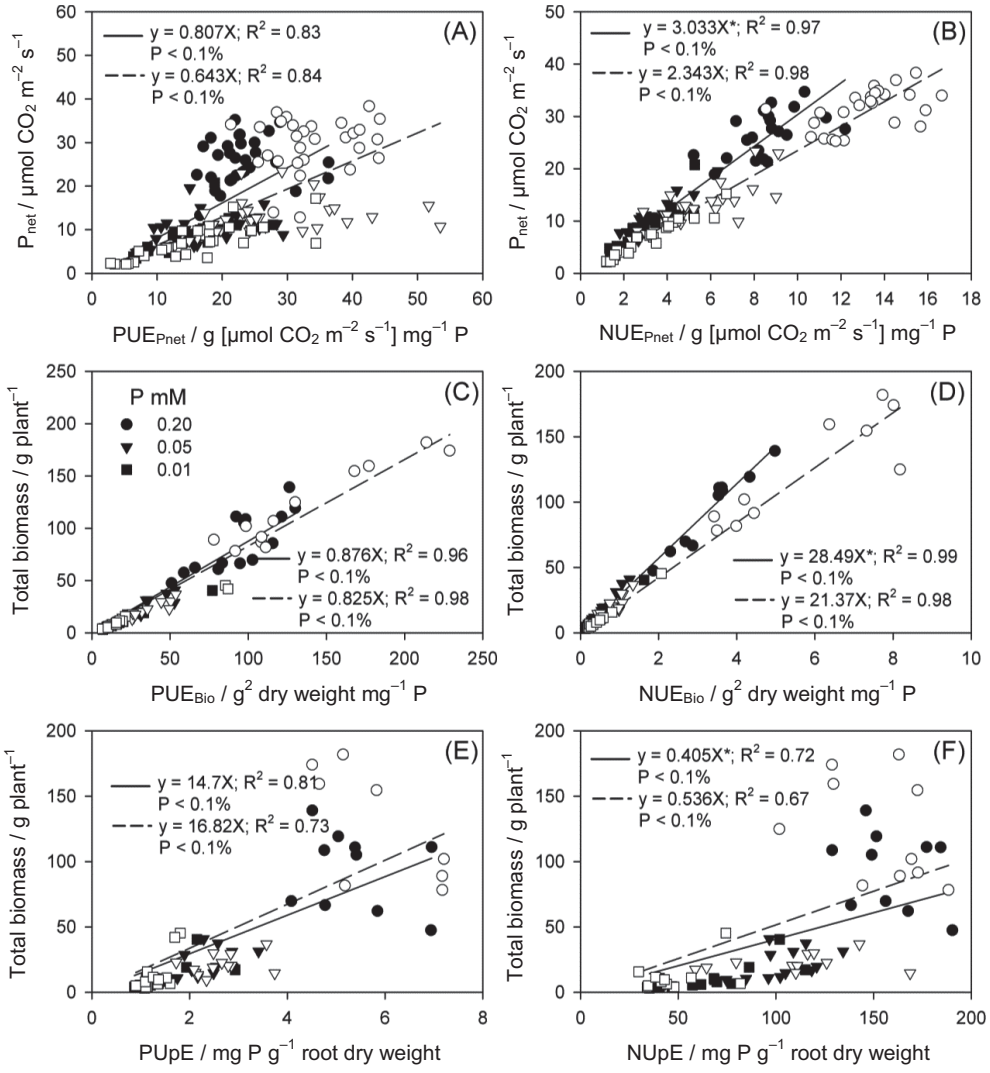


**Figure 6:** The relationship of net photosynthetic rate with the P and N utilization efficiency of photosynthesis ($PUE_{Pnet}$ and $NUE_{Pnet}$, respectively), relationship of plant total biomass with P and N utilization efficiency of biomass production ($PUE_{Bio}$ and $NUE_{Bio}$ respectively) and P and N uptake efficiency of cotton grown at either ambient (filled symbols and solid lines: 400 µmol $mol^{-1}$) or elevated (unfilled symbols and dashed lines: 800 µmol $mol^{-1}$) $CO_2$ under different phosphate (P) treatments. The coefficients of the linear regression analysis are also given, and the intercept was forced to pass through origin (zero). The asterisk (*) indicates significant ($P < 5\%$) difference for the given coefficient between $CO_2$ levels.

The $\Phi_{PSII}$ provides a direct estimate of the efficiency of light use for electron transport by PSII (*Baker* and *Rosenqvist*, 2004; *Singh* et al., 2013a). The low $\Phi_{PSII}$ resulting from the restricted consumption of chemical energy may lead to decreased photochemical quenching which can be detected by reduction in the Fv′/Fm′ ratio as also observed in the current study. The increased $\Phi_{PSII}/\Phi_{CO_2}$ ratio in the P-deficient leaves clearly indicates lower quantum efficiency and electron consumption by processes other than $CO_2$ fixation such as photorespiration, nitrogen metabolism or pseudocyclic electron flux (*Edwards* and *Baker*, 1993). An increased photorespiration under P stress was evident in the current study from the higher values of the $ETR/P_{net}$ ratio (*Singh* and *Reddy*, 2011). Therefore, CF parameters across the P treatments indicate structural changes within the chloroplasts thylakoid membrane. Decreased Fv′/Fm′ and increased Fo′, $\Phi_{PSII}/\Phi_{CO_2}$, and $ETR/P_{net}$ ratio suggest damage to the photosystem functioning in P-deficient leaves.

The response of CF parameters to P deficiency is shown by the significant linear relationship with the tissue P concentration (Fig. 4). The photochemical quenching along with other CF parameters was increased with leaf P, indicating the dependence on P supply for normal functioning of the photosynthetic system. The inverse trend in $\Phi_{PSII}/\Phi_{CO_2}$ and $ETR/P_{net}$ ratios signified an increased requirement of quanta for $CO_2$ fixation with a concomitant increase in photorespiration, respectively. *Jacob* and *Lawlor* (1993) found increased photorespiration in P-deficient sunflower due to changes in the enzyme kinetics favoring oxygenation more than the carboxylation. Thus, CF parameters reflected a marked decline in the photosynthetic efficiency in P-deficient cotton leaves in accordance with our hypothesis. However, marked increases in the CF parameters under $eCO_2$ were not observed, which partly contrasts the hypothesis.

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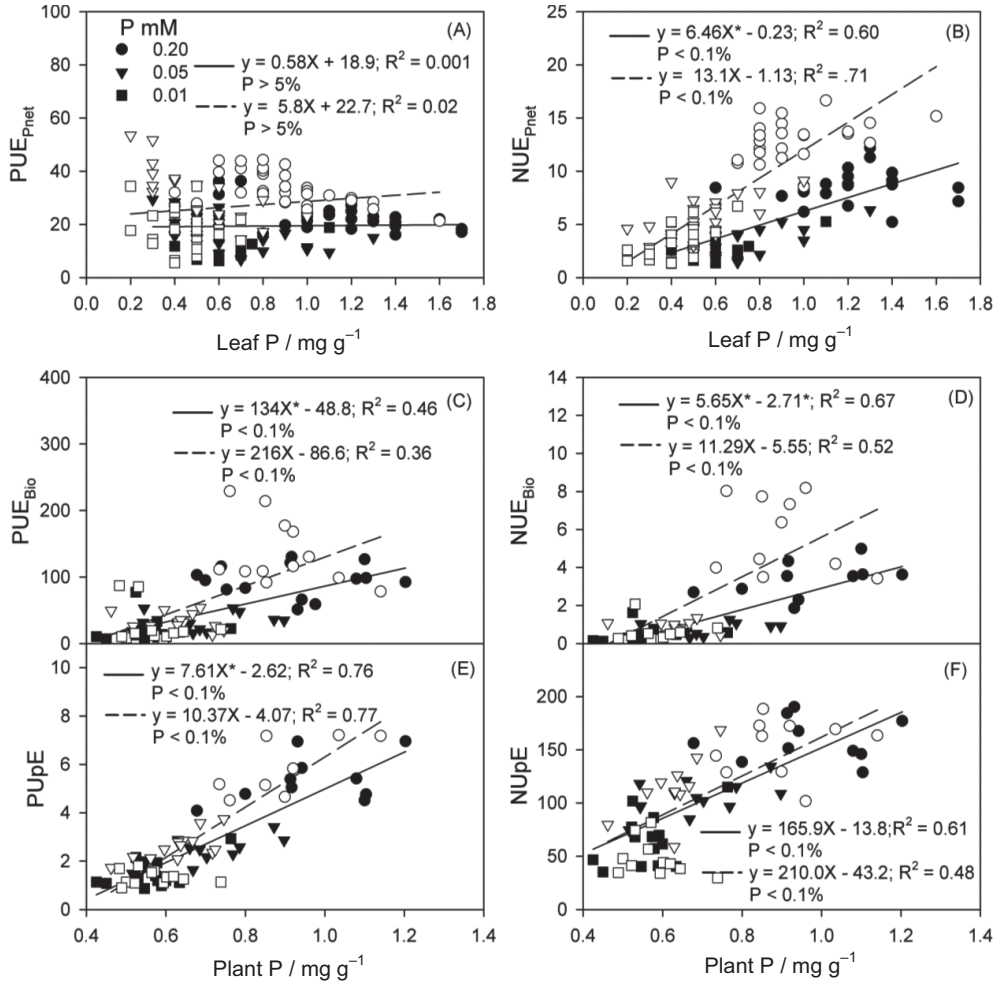


**Figure 7:** Relationship of tissue P concentration with P and N utilization efficiency of photosynthesis [$PUE_{Pnet}$ and $NUE_{Pnet}$, respectively; g ($\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$) $mg^{-1}$ P] and biomass production ($PUE_{Bio}$ and $NUE_{Bio}$ respectively; $g^2$ dry weight $mg^{-1}$ P), and P and N uptake efficiencies (PUpE and NUpE; mg P $g^{-1}$ root dry weight) of cotton plants grown at either ambient (filled symbols and solid lines: 400 $\mu$mol $mol^{-1}$) or elevated (unfilled symbols and dashed lines: 800 $\mu$mol $mol^{-1}$) $CO_2$ under different phosphate (P) treatments. The data are combined across two experiments. For P versus $PUE_{Bio}$, $NUE_{Bio}$, PUpE, and NUpE data are from the final harvests of Experiment 1 (112 d after planting, DAP) and Experiment 2 (91 DAP). The coefficients of the linear regression analysis are also given. The asterisk (*) indicates significant ($P$ < 5%) difference for the given coefficient between $CO_2$ levels.

The acclimation/down-regulation of cotton photosynthetic processes to the long-term $CO_2$ enrichment may be partly caused by imbalance between $CO_2$ fixation and utilization of assimilates (*Barrett* and *Gifford*, 1995; *Singh* et al., 2013a).The $eCO_2$-mediated alteration in the photosynthetic processes may also be adjusted by the distribution of absorbed light energy between photochemical and non-photochemical processes in the chloroplasts. In this study, the extent of changes in the CF parameters was less in $CO_2$ than P treatments. The low sensitivity of CF parameters to $eCO_2$ in cotton has been postulated previously (*Betsche*, 1994). The increase in Fo′ at $eCO_2$ was also consistent across the measured PAR and $C_i$ especially under high P supply (Fig. 1 and 2) and was similar to the observation made in other species (*Conroy* et al., 1990). The significant decreases in the Fv′/Fm′, ETR, and $\Phi_{PSII}$ under the lowest P supply at $eCO_2$ were also apparent from their regression with tissue P concentration and signified a greater damage to the photosystem functioning. Due to the reduction in photorespiration, the energy demand might be lower at $eCO_2$ *versus* $aCO_2$ and this excess radiant energy may also lead to thylakoid energization as indicated by small changes or reduction in qP and other CF parameters (*Betsche*, 1994). The $eCO_2$-mediated reduction in the $\Phi_{PSII}/\Phi_{CO_2}$ ratio and photorespiration as deduced from the lower $ETR/P_{net}$ ratio in $eCO_2$ *versus* $aCO_2$ treatment were also found by *Edwards* and *Baker* (1993) and *Singh* et al. (2013a). This was supported by inverse relationship (negative slope) of tissue P concentration with $\Phi_{PSII}/\Phi_{CO_2}$ and $ETR/P_{net}$ ratios exhibiting lower values at $eCO_2$ (Fig. 4G, H).

## 4.2 Nutrient utilization and uptake efficiency

Contrary to one of our hypotheses, $PUE_{Pnet}$, $PUE_{Bio}$ and $NUE_{Bio}$ decreased due to P deficiency. As discussed below, this might have been caused by the percentage decrease in the $P_{net}$ and TBM in the P-deficient plants. However, the stimulating effects of $eCO_2$ on these parameters were in accordance with our hypothesis, especially in the two higher P-treatments.

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Irrespective of the $CO_2$ treatment, the effect of P deficiency on the P and N utilization and uptake efficiencies was consistent across the harvest dates in both experiments. As compared to the 0.20 mM P treatment, the decline in $PUE_{Pnet}$ and $NUE_{Pnet}$ was highest at 0.01 mM P supply. In contrast, $eCO_2$ increased $PUE_{Pnet}$ and $NUE_{Pnet}$ by 46–63% across P treatments. However, this increment was minimal or none at the lowest P treatment. Under severe P-deficiency, the decline in $PUE_{Pnet}$ and $NUE_{Pnet}$ reflects the lack of the stimulatory response of $eCO_2$ on $P_{net}$, which may be attributed to the increased photoinhibition, photo-biochemical limitation and decrease in photorespiration causing an imbalance in $CO_2$ fixation and recycling of inorganic phosphate in chloroplasts (*Betsche*, 1994; *Singh* et al., 2013a; *Singh* et al., 2013b).

The $PUE_{Pnet}$ was mainly influenced by the photosynthetic capacity of plants as deduced from the significant linear relationship between $P_{net}$ and $PUE_{Pnet}$ (Fig. 6A). However, a stable $PUE_{Pnet}$ across a range of leaf tissue P concentrations was also apparent from its non-significant relationship with tissue P across $CO_2$ levels (Fig. 7A). Although a significant $PUE_{Pnet}$ *versus* tissue-P relationship was not established, $PUE_{Pnet}$ tended to be higher at $eCO_2$. The $P_{net}$ exhibited a close relationship with $NUE_{Pnet}$ which was also significantly increased with an increase in tissue P indicating the dependence of the photosynthetic processes on the N availability.

The linear relationship between tissue P with $PUE_{Bio}$ and $NUE_{Bio}$ ( Fig. 7B, C) revealed that due to P deficiency the biomass decreased more than tissue-P concentration, leading to the marked decrease in P and N utilization efficiencies of biomass production across the $CO_2$ treatments in both experiments. This was also reflected in the decreased P and N uptake efficiencies, in which the total nutrient acquisition per unit of root mass decreased more than the tissue P or N concentration in P-deficient plants. Growth rate, nutrient uptake and tissue concentration play a major role in the determination of nutrient utilization efficiency which may vary among species. For instance, *Cure* et al. (1988) found an increase in P-use efficiency in P-deficient soybean, which might have been caused by a greater decrease in tissue-P concentration in proportion to the biomass of soybean as the P supply was reduced. Moreover, a continued increase in tissue P-concentration, while the maximum growth was attained in the P-sufficient plants, may also cause the decline in the P-utilization efficiency as postulated by *Cure* et al. (1988).

The strong linear correlation between total biomass and the P and N utilization efficiencies demonstrates the relationship between cotton biomass production and nutrient-utilization efficiencies across P supply and $CO_2$ levels (Fig. 6C, D). Plants may be more efficient in nutrient acquisition and utilization under $eCO_2$ due to increased shoot and root growth, and alteration in the physiology including reduced tissue nutrients (*Pérez-López* et al., 2014; *Rogers* et al., 1993). In the current study, $eCO_2$ increased $PUE_{Pnet}$ and $NUE_{Pnet}$ by 46–63% across P treatments. However, this increment was limited to the two high P treatments. Similarly, $PUE_{Bio}$ and $NUE_{Bio}$ were also increased mainly at the two higher P treatments. The lack of $eCO_2$ stimulation under the lowest P treatment of these and other parameters might have also led to the observed $CO_2 \times$ P interactions. For instance, under the lowest P treatments the $PUE_{Pnet}$ was almost similar at both $aCO_2$ and $eCO_2$. Similar inferences can also be made from the relationship between tissue P concentration and other parameters, which indicates a similar response across the $CO_2$ levels when the tissue P concentration was lower (Fig. 4 and 7). Therefore, at very low tissue-P concentration the lack of response in nutrient efficiency and photosynthetic parameters to $eCO_2$ was due to insufficient tissue-P concentrations (*Conroy*, 1992). Previous studies have also reported that cotton plants grown under $CO_2$ enrichment were more efficient in the nutrient retrieval and utilization. However, under severe P stress the response to $eCO_2$ was highly reduced mainly due to severe reduction in growth (*Prior* et al., 1998; *Singh* et al., 2013b).

The N utilization and uptake efficiencies were closely associated with both tissue-P concentration and biomass production, or $P_{net}$ indicating greater dependence of plant growth and photosynthesis on N. Compared to the other nutrients, N is required at the highest amount and directly influences photosynthetic processes and plant growth. The P deficiency-mediated changes in N metabolism have been reported in previous studies (*Fleisher* et al., 2013; *Rufty* et al., 1993). Although N was not limiting in the root media, both treatments (P and $CO_2$) altered N acquisition and assimilation by the plants as revealed by changes in N uptake and utilization efficiencies.

## 5 Conclusions

The CF parameters were affected more by P nutrition than $CO_2$ treatments. A consistent increase in the Fo′ was associated with a decrease in photochemical quenching and other CF parameters in the P-deficient leaves, leading to reduction in the efficiency of energy transfer to the PSII reaction center and quantum yield. The P deficiency consistently decreased P and N uptake and utilization efficiencies for biomass production and photosynthesis. Due to P deficiency, the decreases in the $PUE_{Bio}$ and $NUE_{Bio}$ were mainly attributed to the greater percentage decreases in the biomass production *versus* tissue P concentration. The utilization and uptake efficiencies of P and N tended to show greater response to $eCO_2$ *versus* $aCO_2$, especially at the higher P nutrition. Thus, the beneficial effect of $eCO_2$ on cotton photosynthesis and nutrient efficiency is decreased under P deficiency and might be even negative under severe P deficiency.

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